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HPLC ANALYSIS OF AMINOACIDS IN THE MERCURY AND CADMIUM TREATED GILL, LIVER AND MUSCLE IN *Clarias batrachus*

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ABSTRACT

High performance liquid chromatography (HPLC) based separation of individual amino acids was quite revealing and promising field. It has provided the present study with necessary information on amino acid metabolism in the target tissues of the experimental fish *Clarias batrachus* and also in their relative assimilation, storage and excretion during stress induced response with treated heavy metals like mercury (Hg) and cadmium (Cd). This induction was found to be both as tissue and time specific as it was clearly observed in the morphological changes in gill, liver and muscles. Such differential expression was a great monitor to understand the physiological and cellular adaption's of the fish. The LC50 values were observed at 1.5ppm and 8.0ppm with the treated heavy metals of mercury and cadmium in the fish *C.batrachus*. HPLC studies revealed changes in the intensity and ranges of amide I, II and III bands in control, mercury and cadmium treated tissues. Amide II band was only the characteristic feature of the treated tissues and it was absent in the control tissues. Amide III band was notably present in the liver tissue exclusively. The presence of these amide band results gave a new direction to the studies on binding site determination of heavy metals like mercury and cadmium.

Key words: Mercury, Cadmium, Aminoacids, HPLC, *Clarias batrachus*

MATERIALS AND METHODS

The amino acids differential expression in the stress induced experimental fish *Clarias batrachus* with treated heavy metals Mercury (Hg) and Cadmium (Cd) were quantified using HPLC technique with pre column. Derivatization by O-phthalaldehyde protein assay and with detection at wave length 338nm as described by BRUCKNER et al. (1991) the sample tissue was precipitated with equal amount of 10% TCA. Supernatant collected was used for the HPLC analysis. Equal amount of the sample of 10µl, Internal standard and OPA reagent were blended and the resulting mixture was diluted five times (50µl) with borate buffer of pH 10.2. From this mixture 20µl were injected for further analysis.

RESULTS

Gill

Control gill exhibited highest concentration of Glutamic at 281.24 nm/ml and lowest concentration of phenylalanine was observed at 3.06 nm/ml (Table 1 and Figure 1), while the Mercury treated gill showed highest concentration of Glutamic 485.49 nm/ml and lowest concentration of phenylalanine at 1.83 nm/ml (Table 1 and Figure 2) and the cadmium treated gill also showed highest concentration of Glutamic at 314.89 nm/ml and the lowest concentration of phenylalanine at 2.04 nm/ml (Table 1 and Figure 3). Out of total fifteen amino acids in three tissues, six of the aminoacids Glutamic acid, lysine, histidine, alanine, serine and aspartic acid showed an increase in their concentrations compared to control. The level of phenylalanine acid in mercury

treated gill at 1.83 nm/ml was marginally less compared to the control gill at 3.06 nm/ml and phenylalanine at 2.04 nm/ml in cadmium treated gill. This confirmed the fact that heavy metals, mercury and cadimum considerably suppressed the bio synthesis of Glutamic acid in the gill of *C-batrachus***.** The levels of Glutamic acid, Lycine, Histidine, Alanine, Serine, showed fluctuations in expression levels when compared with control, cadmium and mercury treated gill. Generally mercury treated gill showed more amino acid concentration than cadmium treated gills. The level of Glutamic showed a steep decline in mercury treated gill at 485.49 nm/ml and also in cadmium treated gill at 314.89 nm/ml compared to the maximum concentration in control gill 281.24 nm/ml.

In Liver

In the control liver, highest concentration of glutamic acid at 304.45 nm/ml and lowest concentration of valine amino acid at 4.60 nm/ml was observed (Table 2 and Figure : 4). Mercury treated liver also witnessed highest concentration of the glutamic acid at 326.11 nm/ml and lowest concentration of phenylalanine at 2.24 nm/ml (Table 2 and Figure : 5) and the cadmium treated liver showed with maximum cocentration of glutamic at 303.29 nm/ml and low amount of isoleucine at 2.01 nm/ml acid (Table 2 and Figure : 6). This clearly suggested increased biosynthesis of glutamic acid in the liver of *C. batrachus* exposed to mercury and in cadmium on one hand has diminished the synthesis of glutamic acid. The level of threonine dropped in mercury treated liver of C. *batrachus* compared with their respective control levels. On the other hand there was a significant increase in the levels of glutamic, histidine, lysine, alanine, serine and aspartic acid compared with their respective controls (Table 2, Figure: 5). Cadmium treated liver of *C. batrachus* showed a decreased level of tyrosine, methionine, and Alanine. Glycine Threonine, phenylalalnine and isoleucine compared with their respective controls. Only glutamin and histidine levels were upregulated in the liver treated with cadmium (Table 2 Figure:6).

In Muscles

Table 3 and Figure : 7, 8 and 9 summarises the aminoacid levels in control, mercury and cadmium treated muscle of *C. batrachus* control muscle which exhibited highest concentration of glutamic acid at 916.44 nm/ml and lowest concentration of valine amino acid at 15.42 nm/ml. Mercury treated muscle (Table : 3 and Figure : 8) showed greater concentration of glutamic acid at 377.18 nm/ml while cadmium treated muscle (Table : 3 and figure : 9)showed greater concentration of glutamic at 519.92 nm/ml. Threonine amino acid was not detected in both mercury and cadmium treated muscle of *C.batrachus***.** Out of a total of fifteen amino acids in the muscles, fourteen aminoacids (glutamic, aspartic acid, serine, histidine, glycine, threonine, alanine, arginine, tyrosine, valine, methionial, phenyulalanine, isolencine, leucine and lysine) showed significantly higher concentration levels due to mercury treatment compared to control muscle of *C. batrachus*. Cadmium treated muscle showed higher amino acid levels of twelve amino acids (aspartic, acid, serine, glycine, alanine, arginine, valine, methionine, phenylalanine, isoleucine, leucine and lysine) compared with their respective controls. Only threonine and tyrosine are at undetected levels due to cadmium treatment compared with their respective control (Table : 3 and Figure : 9).

Table 1

Amino acid analysis showing the amino acids level in the Control, Mercury and Cadmium treated Gill in Clarias batrachus

Amino acids	Amino acid level in nm/ml		
	Control	Mercury treated	Cadmium treated
Aspartic acid	26.14	24.18	34.99
Glutamic acid	281.24	485.49	314.89
Serine	31.48	56.37	61.77
Histidine	69.63	190.75	140.34
Glycine	4.11	11.61	17.17
Threonine	0.00	0.00	0.00
Alanine	51.05	119.12	75.78
Arginine	8.26	31.77	13.34
Tyrosine	8.39	22.05	7.45

Valine	6.90	8.98	5.29
Methionine	3.35	12.80	3.66
Phenylalanine	3.06	1.83	2.04
Isoleucine	6.02	9.03	6.02
Leucine	5.25	13.81	7.97
Lysine	253.76	92.48	55.80

Table 2

Amino acid analysis showing the amino acids level in the Control, Mercury and Cadmium treated Liver in Clarias batrachus

Amino acids	Amino acid level in nm/ml		
	Control	Mercury treated	Cadmium treated
Aspartic acid	32.24	35.58	24.96
Glutamic acid	304.45	326.11	303.29
Serine	26.69	88.76	27.59
Histidine	46.06	97.91	62.01
Glycine	0.00	19.83	5.08
Threonine	0.00	0.00	0.00
Alanine	10.66	56.04	44.92
Arginine	36.22	19.70	7.94
Tyrosine	9.63	11.18	5.90
Valine	4.60	3.91	2.30
Methionine	7.92	6.70	3.66
Phenylalanine	0.00	2.24	0.00
Isoleucine	0.00	9.36	2.01
Leucine	0.97	5.83	2.14
Lysine	59.87	70.57	61.15

Table 3

Amino acid analysis showing the amino acids level in the Control, Mercury and Cadmium treated Muscle in Clarias batrachus

Amino acids	Amino acid level in nm/ml		
	Control	Mercury treated	Cadmium treated
Aspartic acid	36.17	21.43	24.18
Glutamic acid	916.44	377.18	519.92
Serine	140.33	54.57	82.16
Histidine	223.75	91.39	137.44
Glycine	55.38	14.51	24.18
Threonine	25.16	0.00	0.00
Alanine	166.53	74.19	99.83
Arginine	109.93	16.84	42.26
Tyrosine	50.00	6.83	23.29
Valine	15.42	6.21	10.36
Methionine	26.81	7.92	13.71
Phenylalanine	17.73	6.72	10.60
Isoleucine	27.09	9.36	15.72
Leucine	52.11	13.42	29.75
Lysine	183.18	78.47	115.16

DISCUSSION

Amino acids are the basic units of a protein molecule and they form the building blocks of protein synthesis (Vonwachledonk and Kappler, 1977). The amino acids are precursors of many bio-molecules, which serve important biological functions, such as hormones, vitamins, Coenzymes, porplusike and neurotransmitter substances (Lehninger, 1993). The present investigation showed that increase in concentration level of amino acids i.e. six amino acids in gill, seven aminoacids in liver and nine aminoacids in muscle out of a total fifteen amino acids present in the freshwater fish *C.batrachus***.** This was correlated and analyzed in confirmation with the other works discussed previously. This might indicate mobilization of aminoacids through proteolysis under the induced stress response in experimental conditions. The undetected levels of Threonine amino acid were in Mercury and Cadmium treated muscle showed mostly a

decreasing trend in gill and liver of the experimental fish compared with their respective controls. Recent studies by Trotti et al., (1997a, 1997b and 1998) demonstrated that glutamic uptake could be altered by agents that react with SH groups on cystenine residues. Methyl mercury also potentially and specifically inhibits glutamate uptake in astrocytes, resulting in excessive concentrations of excretory amino acids (EAAS) in the extra cellular fluid. The brain must synthesize for itself for both glutamate and glutamine because these pivotal amino acids cross the blood - brain barrier very poorly, if at all (smith et al., 1987;Grill et al., 1992). Glutamate can undergo deamination catalyzed by glutamate dehydrogenase producing NH_4^+ and α-Ketoglutacase (campbel), 1991; Hertz et al., 1983; Mckenna et al., 1993). The Latter is then fed into the Kreb's Cycle. Glutamate can also undergo transmisnation with pyruvate, catalzed by alanine aminotransferase, producing α-Ketoglutarate without the release of ammonia. In fish, alanine constitutes 20 - 30% of the total amino acids pool (Hochachaka and Gupp, 1987). Most of the FAAs can be converted to alanine, and the overall quantitative energetic may appear to be quite favourable. The net conversion of glutamate to alanine would yield 10 moles of ATP per mole of alanine formal.

In the present study, mercury treated muscle showed increase in concentration of alanine, which may be accompanied by a decrease in glycogen content. This indicates to the proposition that *C. batrachus* use 5 proteins and aminoacids to support their activities during the stress period. This shift in the metabolic pathway could, therefore sustain the higher metabolic rise (KOK et al., 1999 and Lim et al., 2001). The liver plays an important role in the regulation of a fish's energy metabolism and in directing nutrients to the rest of the tissues (shoemaker and Elwyn, 1969). It is the main site of ammonia genesis (walton and Cowey, 1977), fatty acid synthesis (Henderson and sargent, 1981), and gluconeogenesis (Mommsen, 1986) and because of all these functions liver is involved in the direct regulation of fish growth. The liver contains a heterogenous pool of proteins and as a short - term store of amino acids. The liver of rainbow trout *(Oncor hychus mykiss)* shows high protein synthesis and degradation rates but low efficiency for protein deposition these attributes allow the tissues protein pools to adapt to functional demands on the liver. In the present study, control liver exhibited highest concentration of threonine. The level of alanine though dropped in both mercury and cadmium treated liver which contradicts the findings of (Mommsen et al., 1980). Who observed increases of alanine in the liver and thought to be the major of inter - tissue transport for amino-acid derived carbon.

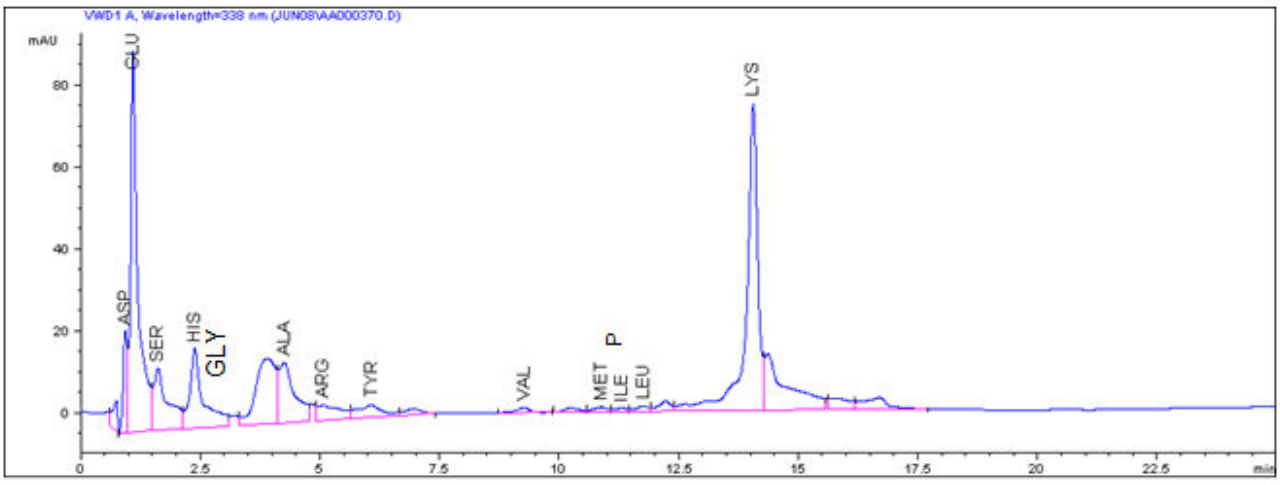

Figure 1
Amino acid analysis by HPLC - Control Gill

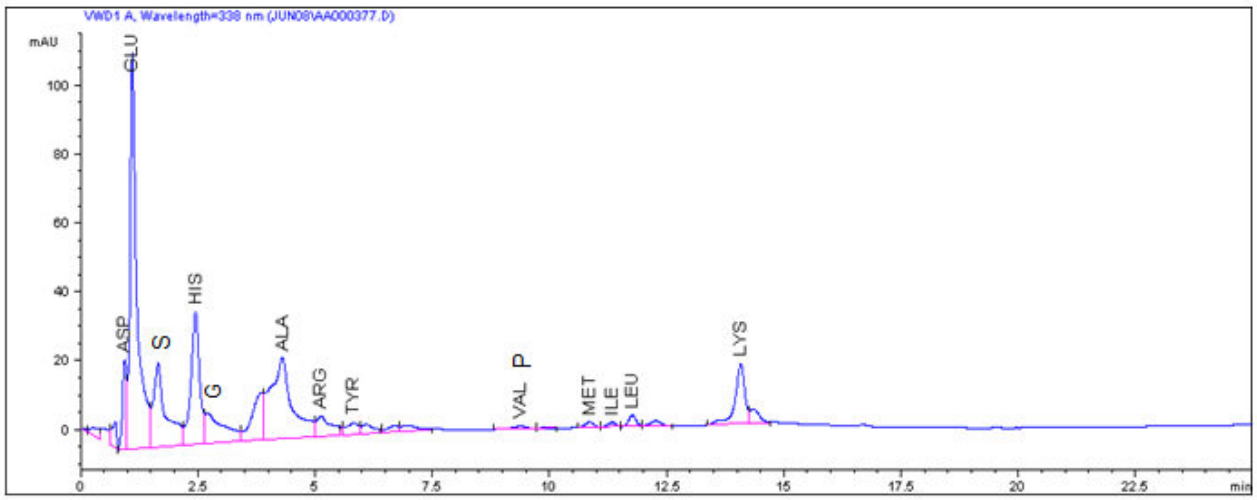

Figure 2
Amino acid analysis by HPLC - Mercury Gill 30 days

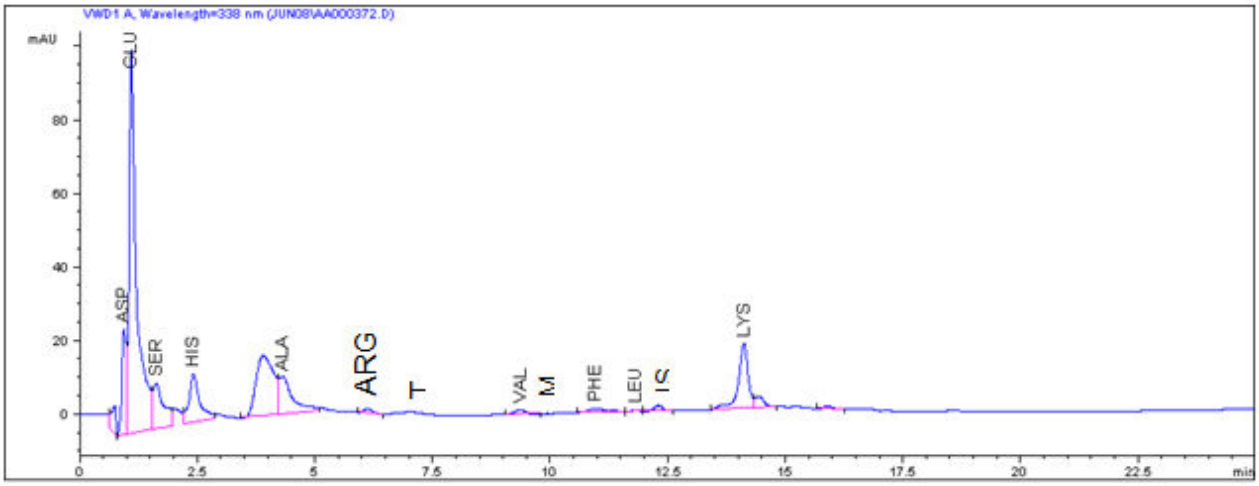

Figure 3
Amino acid analysis by HPLC - Cadmium Gill 30 days

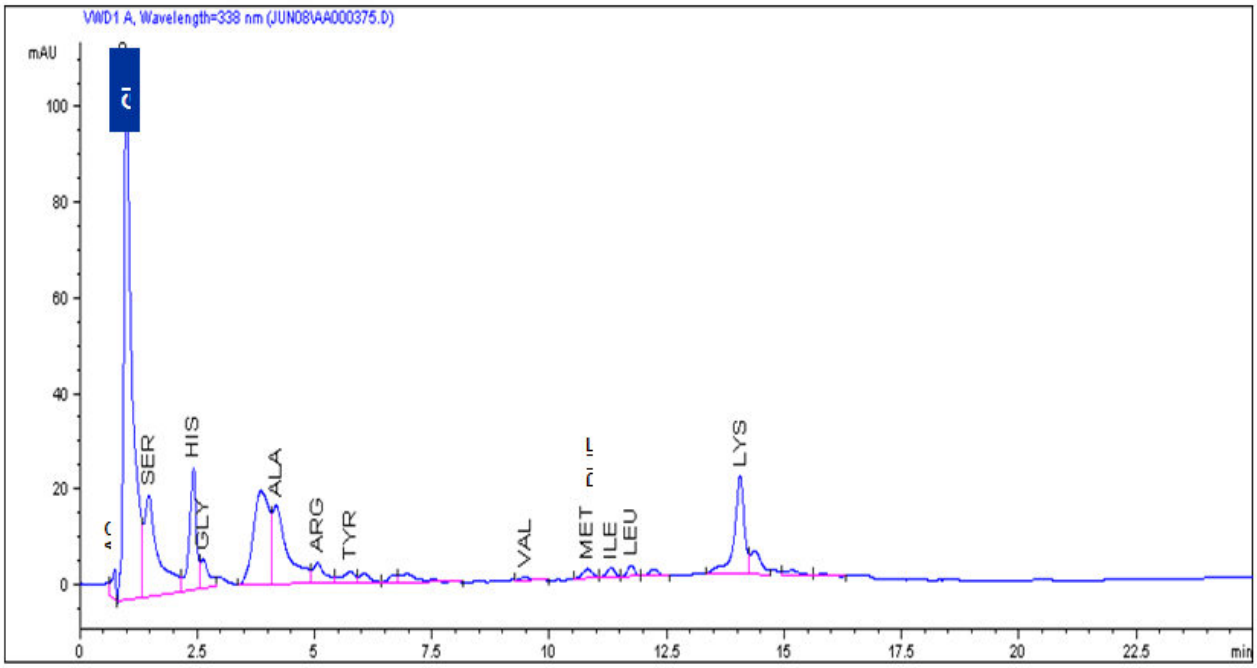

Figure 4
Amino acid analysis by HPLC – Control Liver 30 days

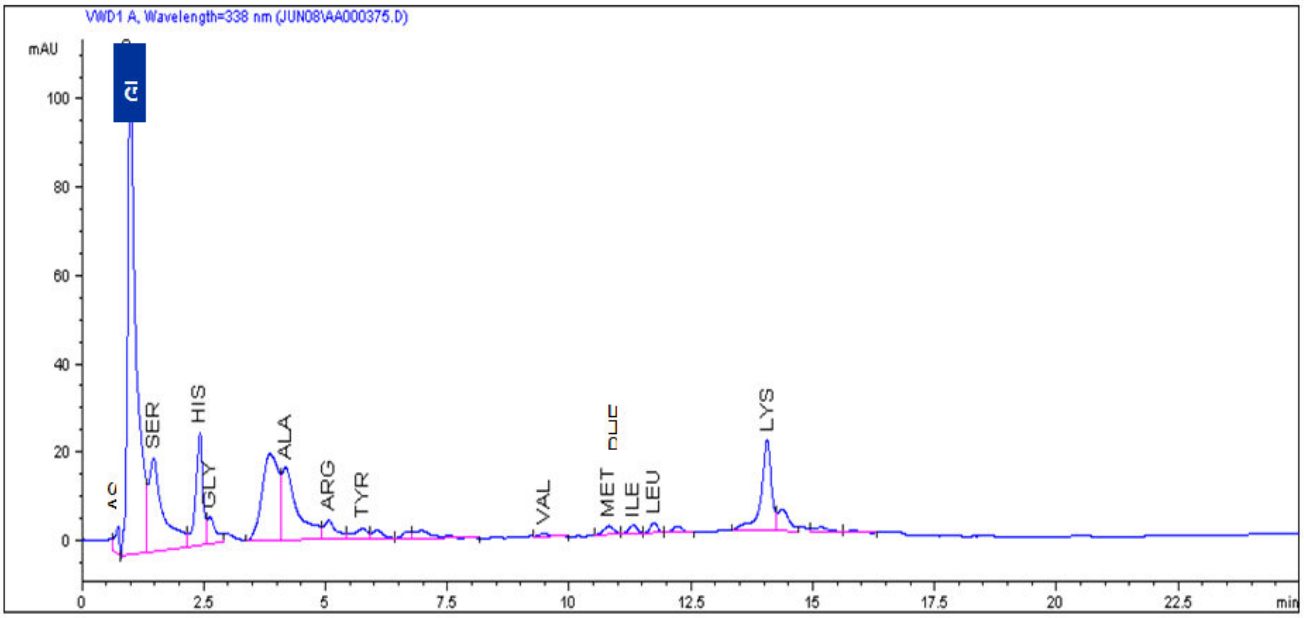

Figure 5
Amino acid analysis by HPLC - Mercury Liver 30 days

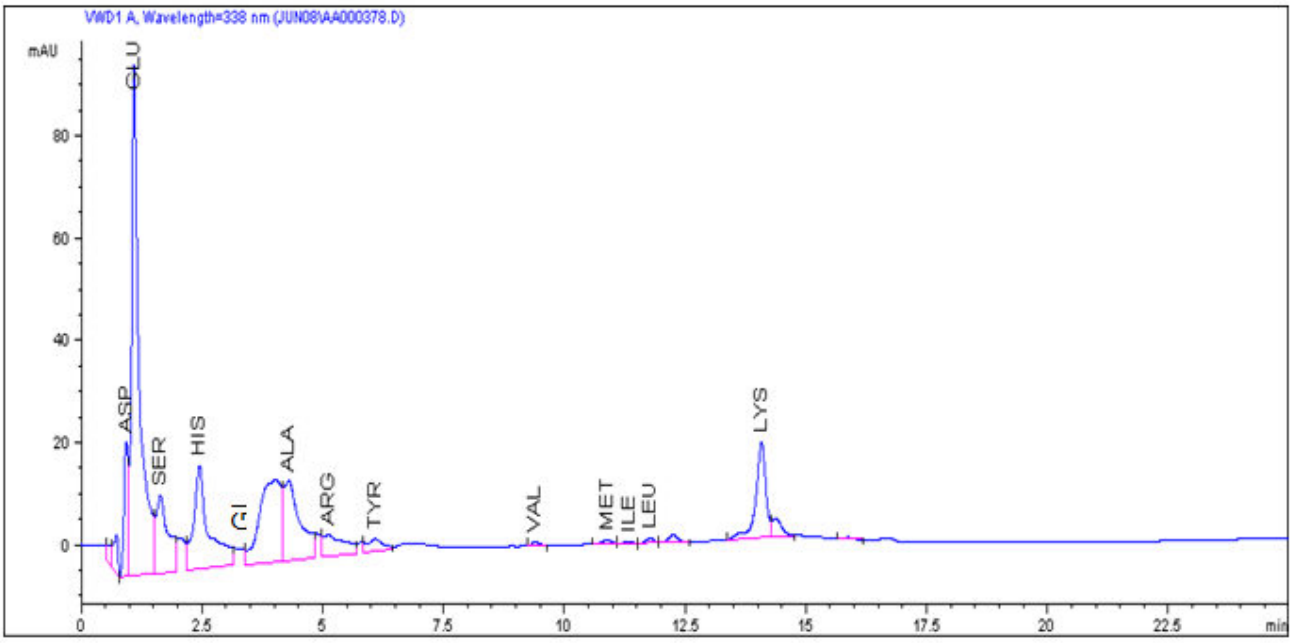

Figure 6
Amino acid Analysis by HPLC - Cadmium Liver 30 Days

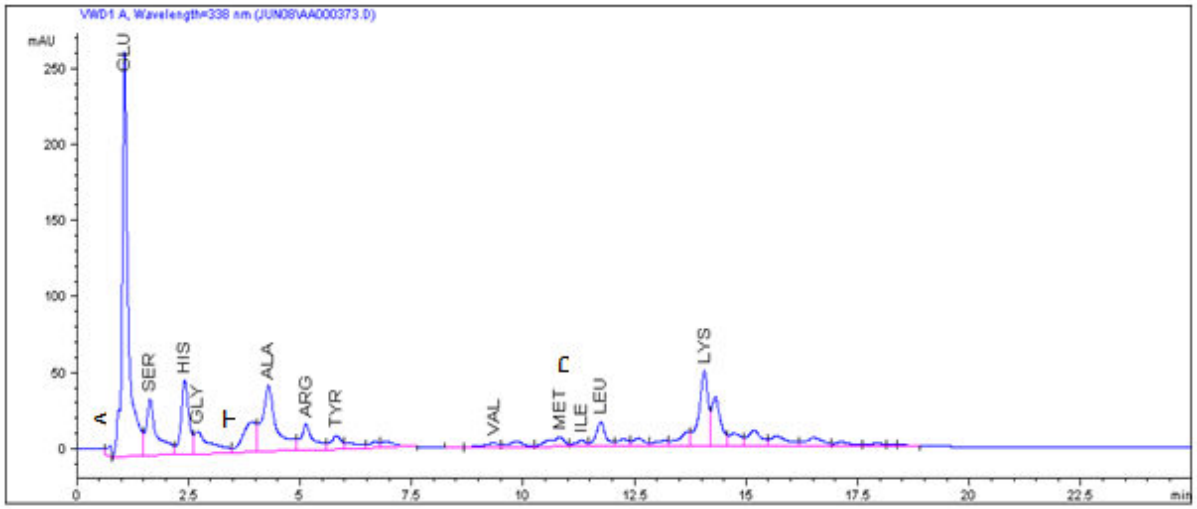

Figure 7
Amino Acid Analysis by HPLC - Control Muscle 30 Days

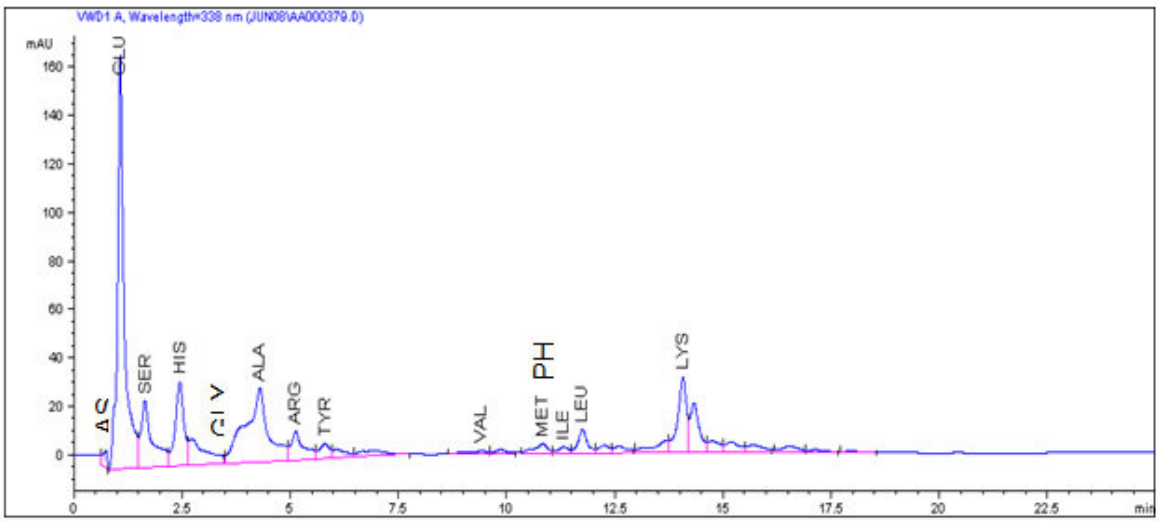

Figure 8
Amino Acid Analysis By HPLC - Mercury Muscle 30 Days

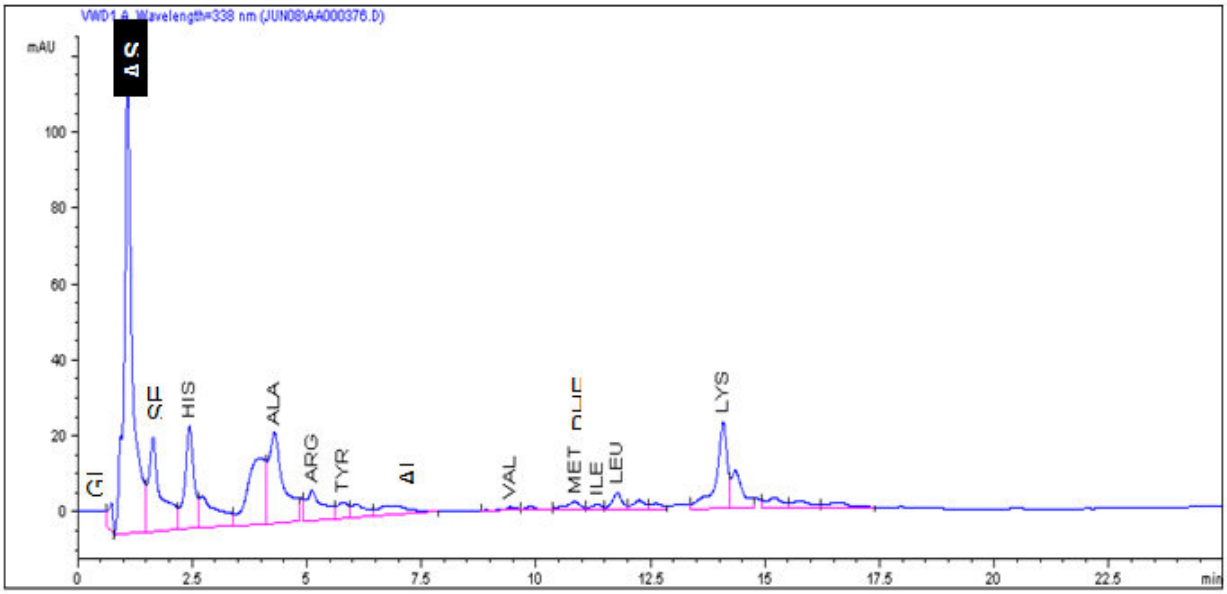

Figure 9
Amino Acid Analysis by HPLC – Cadmium Muscle 30 Days

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